TRANSPORT THROUGH BIOLOGICAL MEMBRANE

DISSERTATION

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In The Loving

Memory

Of My Parents

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This is to certify that the dissertation for M.Phil. entitled **"TRANSPORT THROUGH BIOLOGICAL MEMBRANE"** embodied the original work carried out by **MR. NISAR HASAN KHAN** under my supervision. He has fulfilled all the requirements for the degree **"MASTER OF PHILOSOPHY"** in Chemistry, regarding the nature and period of investigational work.

(DR. HASAN ARIF)

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Nisar Hasan Khan

NISAR HASAN KHAN

C O N T E N T S

LIST OF TABLES

CHAPTER - I

I N T R O D U C T I O N

The steadily detoriating conditions of human life in civilised areas have much diminished the numbers of those who once considered the chief aim of science to be the unlimited subjugation and transformation of nature. The main importance of science, of course, never lay in this area and in the process of seeking a more thorough understanding of nature. Scientists have constantly imitated nature specially living nature. This direction appears even more promising at the present time. Since the artificial tools, materials or processes developed are not only useful in themselves but in addition provide models which on investigation provide a deeper understanding of the natural phenomena.

For along time transport phenomena in membrane could not receive due attention as it deserved. This is perhaps due to non-industrialisation and lack of contact among scientists from related branches. From last few decades with the development in industries it has gained considerable significance because of its use in several techniques like full cell technology etc. It also has direct impact in desalination, medicinal biology, and several other processes. The scientists from all related branches; electrochemistry, biophysics, biology, chemical engineering etc. have done significant work and it resulted in the collection of enormous literature in this field.

Many kinds of membrane processes have been proposed theoretically, and some of them are now becoming commercially feasible processes such as reverse osmosis, ultrafiltration, dialysis, ion-exchange, gas separation and so on.

Kobatake et. al. (1-3) and Nagasawa et. al.(4) developed certain theories of membrane potential, based on non-equilibrium thermodynamics, for synthetic membranes such as ion-exchanger membranes, bilipid layer membranes etc., prepared in laboratories from various chemical substances. The transport of ions across these artificial membranes were found to be governed by the laws of irreversible thermodynamics. Since the ultimate aim of fundamental research is its application in the service of mankind, it is natural to examine the applicability of these laws of irreversible thermodynamics to biological system.

In the present work, special emphasis has been laid to study certain biochemical and thermodynamical properties of pericardial membrane, based on the laws of irreversible thermodynamics by utilising different theories put forward by several scientists.

The experimental set up has been shown in fig. 1 and explained under the heading "experimental".

In the forthcoming topics of this dissertation I have mentioned briefly the function and structure of biomembrane, particularly, pericardium. The studies on biomembrane are important in every sphere of clinical medicine. The characterisation of the composition of biological membrane and its behaviour will be of great clinical significance. Aging process, malignancy, cardio-vascular structure and functional integrity are some of the processes directly related to the biomembrane function.

The biological membranes covering various cells, tissues and organs, govern the movement of nutrients, toxins and other substances across the membrane. Obviously studies on paricardial membrane will definitely form a better model for studies on biological system and such studies will definitely throw light on the behaviour of biological membrane as compared to artificial membranes.

This significance of biological membranes has attracted the attention of physical chemists to examine the applicability of the laws of irreversible thermodynamics developed for artificial membrane to the biological system.

A review of literature has shown that stray attempts had been made in this direction by using frog skin as experimental model by Ussing (5-7). But frog skin was too thick to be a suitable model for such studies. Attempts at

isolation of egg shell membrane had failed because the chemical treatment of egg shell to isolate membrane adversely affected the properties of membrane itself. These thermodynamic studies on biological membranes were of preliminary in nature and suffered from drawback of being quite a thick membrane which caused hindrance in the proper investigation. But, membranes like pericardium, duramater and peritonium obtained from buffalo (bof-bubalis) are thin structure and suit the experimental set-up developed by Kobatake et. al. (1-3).

Studies (8-12) have shown that the laws of irreversible thermodynamics developed by physical chemists for artificial membranes are also applicable for biological membranes such as pericardium, duramater and peritonium. The main function of peritonium is the transport of the fluid and electrolytes, pericardium has supporting and other functions while duramater performs mainly the function of protection of brain. Pericardium and duramater have received great importance during the last decade because of their usefulness in the production of cardiac valves, as the bioprosthetic valves are expected to prove superior to artificial valves because they do not require long term anticoagulant therapy. Such valves are cf greater significance in our country because of lesser financial implication in their manufacture and maintenance. Peritonium

has much importance in peritoneal dialysis in the treatment of patients suffering from renal failures. Furthermore these membranes are thin and no damage is caused during the isolation process.

What I wish to emphasize is that, in spite of greater clinical significance of these membranes, the direction has not been given due attention so far. Previous studies have shown that biophysical and thermodynamic properties are very important in the context of cell membrane. It has now been established by appropriately designed experiments that energy characteristic of cell membrane exerts profound influence on its function by affecting the movement of ions and other toxic substances across the cell membrane. Any change in the structure and function of a membrane will adversely affect the integrity of the cell and ultimately, that of the living organism itself. Unfortunately laws of irreversible thermodynamics which are expected to influence the critical function of cell membrane could not be under taken because of want of unappropriate experimental model as has been discussed above.

In the present study we designed and characterised a model for the study of membrane resistance (Rm), membrane capacitance (Cm), membrane conductance (Cc) and impedance(Z)

across precardial membrane of buffalo (buf-bubalis) aged between 18-24 months. During the course of our studies we found that the biological membranes are anion selective and positively charged in contrast to artificial membranes which are cation selective and negatively charged.

We have also described the difficulties and limitations of our experimental model, but the results are significant and we hope that the findings of this work will form the basis for further research on this important and interesting field of irreversible thermodynamics with respect to biological membranes. The results have been critically analysed under the heading Results & Discussion.

CHAPTER - II

REVIEW OF LITERATURE

2.1 MEMBRANE:

A precise and complete definition of the word membrane is difficult to make (14) and any complete definition given to cover all the facets of statement will be incomplete. In precise and simple terms "a membrane is a phase usually hetrogeneous, acting as a barrier and supporter to the flow of molecular and ionic species present in the liquid and/or vapour contacting the two surfaces". The term hetrogeneous has been used to indicate the internal physical structure and external physico-chemical environment (15-20).

A 'membrane' according to a useful definition, is a solid or liquid film or layer with a thickness which is small compared to its surface.

In case of ion-exchanger membranes, a broader definition has come into use. It includes any ion-exchange material, irrespective of its geometrical form which can be used as a separation wall between two solutions. Many common ion-exchanger membranes are planer disks about 1 mm in thickness. However cylindrical plugs (21,22) or single loads glazed into frames (23) are also called membranes.

One very important and fundamental contribution to the study of membranes came from Donnan (24) whose pioneering work on membrane equilibria has given quite new dimensions to these studies.

Ostwald (25) in 1980 founded the electrochemistry of membranes by considering the properties of semipermeable membranes, that is, one which permits diffusion of certain molecules or ions and restrics diffusion of others.

Membranes of varying degree of permeability and semipermeability occure universely in plant and animal organisms, constituting there cne of the fundamental devices which regulate the exchange of material and, thus, the flux of life. Membranes can thus be classified into two types as artificial and biological membranes.

2.1.1 Artificial Membrane:

Ion-exchanger membranes combine the ability to act as a separation between two solutions with the chemical and electrochemical properties of ion-exchangers. The most important of these are the pronounced differences in permeability for counter ions, co-ions and neutral molecules, and their high electric conductivity. Two different types of ion-exchanger membranes are in use. They are often called "homogeneous" and "hetrogeneous" membranes. "Homogeneous membranes are coherent ion-exchanger gels in the shape of disks, ribbons etc. Their structure is that of the usual ion-exchanger resins. They are homogenous only in dimensions which are large as compared to the mesh width of the matrix. Hetrogeneous membrane consists of colloidal ion

exchanger particles embedded in an inert binder (polystyrene, polyethylene, wax etc.). Their mechanical stability is superior but electrochemical properties such as conductivity and barrier action are not as good as those of the homogeneous membranes (26). For scientific investigations homogeneous membranes have been prefered in the past because of their more uniform structure.

(a) Studies on Artificial Membranes: Transport phenomena such as permeation and membrane potential across synthetic membranes have received a great deal of attention in the last two decades, becasue of its umpteen importance in elucidating the permeability of ions through biological membranes. Consequently, it is necessary to study the model membranes which mimic some of the properties of biomembranes. There are also biologically important substances which resembles the membranous structure of the living cell membrane. Attention has been paid to focus on these structures in order to determine their properties like permeability, membrane potential, conductivity etc.

A number of electrochemists and scientists from other related branches prepared artificial membranes from various substances by different techniques to study the transport phenomena. Liquiri et. al. (27-29), Hays (30), De Korosy (31), Lakshminarayanaiah and Siddiqui (32-34),

Siddiqui et. al. (35-39), Beg et. al. (40-44) prepared complex membranes.

Mueller and Coworkers (45) obtained, for the first time, very thin artificial biomolecular black phospholipid membranes. These phospholipid membranes have been used as a model by various investigators.

Membrane phenomena is so vast that it can hardly be described. Important work in this field is done by Helfferich (46), Marten (47), Marensky (48), Spigeler (49,50), Hope (51), Plonsy (52), Lakshminarayanaiah (53,54), Cole (55), Kimura et. al. (56), Koh and Silverman (57), Srivastava (58,59), Singh et. al. (60), Harris (61), Schlogi (62), Bitter (63) and Keller (64). Kobatake and Coworkers (65-68) have derived an equation from membrane potential data and fluxes from the salt, for the charge density, mobilities and selectivity coefficients of ions. Demish and Pusch (69) has calculated the membrane potential and resistance for binary and ternary electrolyte system in ion-exchange membranes. Minoura and Nakagawa (70) has also measured the membrane potential of poly (d -aminoacids) membranes. Kinoshita et. al. (71) studied the salt permeabilities and membrane potentials of charged polypeptide membranes. Vink (72) also measured the membrane potential and diffusion of sodium chloride in cellulose

membranes and interpreted the results, using modified Nernst Plank equation.

Several theories have been put forward by electrochemists and scientists from other related branches to account the transport phenomena in artificial membrane. Michaelis (73) and his coworkers in twenties and early thirties has tried to characterise the permeability of dense membranes in terms of electrical potentials measured in a solution-membrane-solution system.

Teorell (16) and later Meyer and Sievers (17) independently put forward identical theories which assume that the membrane itself has a fixed charge due to either adsorption or dissociation.

2.1.2 Biological Membrane:

A natural membrane existing in the living system is called biomembrane. All living systems have compartments separated from each other and from external environment by membrane and other barriers. A bacterium, the plasma, a neuron, the whole brain, a mitrochondrion are examples of such compartments. Each compartment maintains a characteristic steady state compopsition usually different from the composition of its surroundings. These membranes are inhomogeneous and exceedingly complex in nature. They

are made up of lipids in the form of bilayer with proteins present at the surface as well as inside the membrane. At some locations thickness of the membrane is of the order of 50-100°A. The characteristic feature of membrane is their function as selective barriers i.e. their selective permeability to various species.

In recent years considerable attention has been devoted to applying the methods of irreversible thermodynamics to flow through biological membranes.

If one examines any real biological system in greater detail, it becomes clear that it is a non equilibrium system. The various carbohydrates, proteins and lipids all co-exist with molecular oxygen, where as equilibrium would emply conversion to carbondioxide and water. Electrical charges are separated across membranes and unequal concentrations are maintained across membranes in living cells. These are also non-equilibrium conditions. The living animal continually maintains body posture and position in a mechanical non-equilibrium state. Plants promote non-equilibrium by storing energy in the sun's rays in the form of sugars. Thus the study of living systems strongly suggests that a theory of non-equilibrium thermodynamics would be useful (74).

2.1.2 (a) Composition and Function of Biological Membranes: The structure of cell membrane is widely recognised as an outstanding problem of present day molecular biology. Uncertainty about the structure of membranes naturally emplies uncertainty about the many mechanism associated with membrane functions. Explanation for transport of ions and molecules, the detail of nerve pulse at molecular level and the way in which energy transports the molecules remains incomplete (4). Biological membranes have many functions. They act as a permeability barrier and transfer matter and information across the boundary between the exterior and interior phases; they can be excitable, they serve to give each cell its own individuality and they act as support for catalytic functions.

The organisation of complex structure cf biomembranes is related to their divergent and specialised functions. In the metabolism of living cells, the role of individual and molecular constituents in the oranisation of structure and functions of membranes and membranous organelles are of prime importance in membrane biology. Informations available at present indicates that proteins of any one type membrane are hetrogeneous. For example Kiehn and Holland (75) have shown that mammalian cell membranes from several sources contain a large number of proteins of different molecular

weights ranging from less than 15,000 to over 100,000. For any given type of membrane; the distribution of protein has found to be quite similar but it differs between different membranes. No single protein component has been found to predominate, although a predominant species might have been expected on the basis of the hypothesis of Green and his coworkers (76) that a "structural protein" is principal constituent of a membrane.

A number of these functions have not yet been assigned to specific components of the membrane. However it is sufficiently clear that proteins with their interrelation with lipids perform most of the dynamic activities of the membrane.

Biological membranes are mainly composed of lipids, proteins and carbohydrates in variable proportions and sugar residues attached to either lipid or protein components or both. These membranes surround the cells and organelle. The cells along with their various organelle constitute a fundamental unit of biological activity and the activities of the cells are governed mainly by the integrity and function of membranes surrounding the cells and its organelle. Thus membranes serve not only as the barrier separating aqueous compartments with different solute compositions but also act as the structural base to which certain enzymes and transport systems are finely bound (77).

The most satisfactory and favourite model of membrane structure today appears to be 'Fluid Mosoic Model' postulated by Singer and Nicolson (78). The essence of their model is that 'membranes are two dimentional solution of oriented globular proteins and lipids'. The major features of this model are:

(i) Most of the membrane phospholipid and glycolipid molecules are in bilayer form. This lipid bilayer has dual role. It is a solvent for integral membrane proteins and it is also a permeability barrier.

(ii) A small proportion of membrane lipid interest specifically with particular membrane protein and may be essential for their function.

The lipids (especially phospholipids and cholestrol) are arranged in bilayer structure so that their polar hydrophilic water soluble portions are separated by hydrophobic regions. The lipid layers are two dimensional liquids in which lipid molecules are laterally moving in monolayer. This lipid from one polar end can not move easily to the other polar end. This flip-flop movement is very slow because polar lipid can not easily cross the non-polar hydrophobic region.

Some recent findings of Hirata and Axelrod (79) have shown that there are methyl transferenses in the membrane which methylate the phospholipids, and methylated

phospholipids can easily translocate from one end to the other, thus reducing the viscosity of membrane to allow the passage of signals.

In the lipid bilayer the proteins are arranged in mosaic fashion which have been divided into two broad categories. Intrinsic or integral proteins are associated with membrane in a line and permanent fashion. The intrinsic proteins are oriented in such a manner that their hydrophobic aminoacids burried in hydrophobic region of lipid bilayer and polar acids on the surface. Thus, same type of integral proteins acquire one configuration. The other category of proteins are extrinsic or periphebral proteins showing a weaker association (80-83).

Rouser and co-workers (84) have summerised their considerable analysis of many membrane systems as follows:

(i) All animal cell membranes contain phospholipids. The same classes of phospholipid are found in verteberates and invertebrates. Some membranes (e.g. myelin) contain glycolipids whereas others do not. Only certain membranes contain sterols.

(ii) Plasma membranes, cell surface of the endoplasmic reticulum, nuclear membranes and mitochondrial membranes from the same species have different compositions all differ quantitatively and to some

extent qualitatively in the classes of lipid present. For example plasma membranes or elaboration of these appears to contain most of the glycolipid of the cell.

(iii) The properties of the different phospholipids vary greatly and the total amount as well as the types of both ceramide polyhexosides and gangliosides are very different species. Data from whole organs indicates that the plasma membrane from different cell types of the same species may vary in composition.

(iv) The fatty acid composition of each class of lipids from different organelles and organs of one specy as well as from different species is variable. This is true even when the classes of lipids are the same in different structures. Individuality is thus expressed most clearly in differences in fatty acids.

2.1.2 (b) Thermodynamical Studies on Biological Membranes: The applied significance of biological membranes has attracted the attention of physical chemists to examine the applicability of laws of transport developed for artificial membranes to the biological system. The membranes are believed to have a fundamental unit membrane structure which

is a biomolecular leaflet of lipid with their polar groups oriented towards two aqueous extra cellular and the intra-cellular phases of the cell. Protein is supposed to exist in close proximity of the polar heads of the leaflet (85,86). Thermodynamical studies on biological membrane have acquired much importance during the last few decades because of the applied significance of biomembranes. Extensive work is available on behaviour and properties of biological membranes. The transport across biological membranes offers specific experimental and conceptual models of interest to the physiologists, the enzymologists, the pharmacologists, the chemists, the biochemists, biophysicsts and the membranologists.

The work is too extensive to be described here. The expensive literature pertaining to membrane phenomena has been reviewed in a number of publications (2-4), significant work in this field is done by; Bonting (87); Heiz (88); Torres et. al. (89), De Villarde et. al. (90), Nakagaki et. al. (91), Ussing (5,6), Zeevi et. al. (92), Simons (93) applied the methods of irreversible thermodynamics to the problem of particle flow through biomembranes.

The general features of carrier transport systems, both equilibrating and transporting uphill were reviewed by Wilbrandt and Rosenberg (94), Katchalsky et. al. (95,96)

elaborated their description of biological transport processes in terms of irreversible thermodynamics.

Walser and Walser et. al. (97) measured the flux of sodium and chloride ions across toad bladder in the absence of ouabain. Chen and Walser (98) also measured the flux of Na and sulphate ions in toad bladder treated with sufficient ouabain to inhibit active sodium transport, at the potential between 0 to 100 milli volts.

Shukla and Mishra (99,100) have carried out the thermodynamic permeability, electro-osmosis permeability and streaming potential measurement for urea and urine solution across urinary bladder membranes based on the method of non-equilibrium thermodynamics.

2.1.2 (c) Pericarium: It is the outermost covering of the heart. It is normally found in the verberates form the lower forms to man (101) and has studied in a wide variety of mammals. It has been compared to a balloon in which the heart is plunged as through it were a first turning and continually changing its configuration during each pumping cycle. These configurational changes involves extensive movement of the first relative to the balloon and this analogy further serves to illustrate the higher surface loading at knuckles simulating transient propusions of the pericardium as they distend pericardium over those areas (102).

2.1.2 (c-i) Composition of Pericardium: Pericardium is composed of an outerlayer, the fibrous pericardium, and an innerlayer, the serous pericardium (103). The fibrous pericardium is connected to the diaphgram by a loose fibrous diaphgramatic attachment and to the sternum by the sterno pericaridal legaments. The serous pericardium consists of a parietal and visceral layer. The parietal layer, is contiguous with the fibrous pericardium and is separated from the visceral layer, which covers the muscular wall of the heart, by the pericardial cavity. The soft collagenous tissues consists predominantly of collagen, elastin ground substance and water. Collagen is a polypeptide chain which when organised into fibers and it is thought to dominate the structural integrity and gross mechanical behaviour of tissues. Elastin is a globular rather than helical protein. Four lysine-derived units join to form four-pronged desmosine linke that ties four elastin polypeptide chains together. The ground substance consists of mucopoly saccharide, glycoproteins and soluble proteins and accounts for less than 1% of the total tissue weight. Bovine pericardium in its natural state consists of 76% by weight of water (104). Almost all of this water is unbound.

2.1.2 (c-ii) Pericardium and Bioprosthetic Valves: The role of pericardium in the production of bioprosthetic valve is of great significance (105,106). These are constructed from

the combined outer layer of serous pericardium and the fibrous pericardium. Bioprasthetic valves are proved to be superior to artificial valves because these do not require long term anticoagulant therapy.

2.1.2 (c-iii) Function of Pericardium: Under normal conditions the percardium, with its fluid lubricates the moving surfaces of the heart, holds the heart in a fixed geometric position and isolates the heart from other structures in the thorax, thus preventing adhesions and spread of infection. It also serves the following functions:

(i) Prevents dilatation of heart chambers and insures that the level of transmural cardiac pressures will be low, never exceeding a few mm of mercury (Hg),

(2) Prevents hypertrophy of the heart under conditions of increased left ventricular outflow resistance,

(3) prevents ventriculo-atrial regurgitation under conditions of increased ventricular end diastolic pressures,

(4) in association with the lungs and tissues surrounding the pericardium, it facilitates the filling of the atria by the development of negative pericardial pressure during ventricular systole,

(5) responds to nerve stimulation and reflexly affects blood pressure and heart rate; and

(6) in association with pleural fluid constitutes a hydro-static system that automatically applies compensating hydrostatic pressures on the outside of the heart when gravitational or inertial forces acting on the heart are altered during acceleration, for example . the automatic hydrostatic compensation insures that end-diastolic transmural pressure is the same at all hydrostatic levels of the ventricle; as a result the stretch of the muscle fibers is uniform and the frank starling mechanism operates equally at all hydrostatic levels within the ventricles.

Functions that various investigators have attributed to the pericardium include prevention or over dilatation of the heart (107-109), protection of heart from infection and from adhesion to the surrounding tissues, maintenance of the heart in a fixed geometric position within the chest (110) regulation of interrelations between the stroke volumes of the two ventricles, and prevention of right ventricular regurgition when ventricular diastolic pressures are increased (111,112).

2.2 STUDIES OF THERMODYNAMICAL PARAMETERS (Capacitance, Resistance, Conductance and Impedance) ACROSS MEMBRANE:

Capacitance and resistance are very important parameters for the study of many electrochemical systems. In

order tc understand the behaviour of complex biomembranes, simple polymeric (113), liquid bilipid layer membranes (114,115), parchment (116-119) and millipores (120-122) filter paper supported membranes, in recent years have been used as a model by a number of investigators. Warbury (123) developed the theory of diffusional impedances and derived the expression for it. Impedance measurements provide a powerful diagnostic tool for the analysis of many electrochemical systems (124-127). The work of Macdonald (128) provides a systamatic treatment of small signal a.c. responce of conducting cell and membranes. Archure and Armstrong (129) interpreted the complex impedance spectra for solid electrolyte interfaces. F.A. Siddiqui and Coworkers observed resistance of cadmium hexacyanoferrate (130) and Parchment supported ferric molybdate (131) artificial membranes, equilibrated with different electrolytes, with same concentration and found that resistance increases in the order KCl, NaCl & LiCl for electrolyte (1:1), $CaCl_2$, $MgCl_2$, $BaCl_2$ for electrolytes (2:1) and electrolyte (3:1) $AlCl_3$ produces the highest value of membrane resistance. Takashima et. al. (132) has shown that the parallel resistance and capacitance may transform to a series arrangement. A.E. Hill (133) while studying ion transport through leaf gland cells of limonium measured the impedance characteristic of the whole leaf disk. The frequency characterisitic was examined with a.c. bridge

circuit. The a.c. responce to a rough electrode surface in contact with an aqueous electrolyte has been discussed by de Levis (134,135). Bhomic, W. and his coworkers (136) measured electrical conductivity of electrolytes such as HCl, LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, $ZnCl_2$ having common anions but different cations with concentrations ranging from 0.1 to 0.2 mM across oxidised cholestrol bilayer lipid membrane both in presence and absence of iodine and reported the following order $Li^+ > K^+ > Ca^{++} > Na^+ > Mg^{++} > Zn^{++} > H^+$. They also reported that with the increase in the concentration of salts electrical conductivity increases and attains a saturation value just above 1 mM in all cases. The technique of capacitance has been applied to many passive and excitable membranes (137-140). To demonstrate the effect of H^+ on membrane resistance, H.U. Demisch and W. Pusch (69) observed membrane resistance for the system HCl-LiCl, $MgCl_2$-HCl and $MgSO_4$-H_2SO_4 with equal concentrations of solution on both the sides of membrane, at different but constant pH values. They observed that membrane resistance exhibits a distinct maximum at intermediate salt concentrations and maximum resistance decreases with decreasing pH. They explained the decrease in the resistance with decreasing external salt concentration as due to the exchange of H^+ against the cation. Early works of Cole and Curtis (137) and Cole and Baker (141,142) demonstrated

various unique features of nerve membrane. The analysis indicates that the effect of electrolyte concentration and bridge frequency on membrane capacitance is not due to electrode polarisation but may be due to some structural changes in the membrane as discussed by Chandler et.al. (143). The frequency dependance of electrical impedance of a tissue is conveniently represented by the impedance locus. A photograph of reactive component against resistive component with frequency as implicit parameter. The impedance locus of a cell membrane is frequently a circular arc with its centre below the resistance axis. M.N. Beg et.al. (43,44) observed specific conductance of chloride (1:1) salts across parchment supported cupric orthophosphate membrane and parchment supported thallium dichromate membrane and reported almost linear increase in the specific condutance with the square root of concentration of bathing electrolytes solution. He also observed that specific conductance attains a maximum limiting value. S.K. Srivastava & coworkers (144) measured the conductance across polystyrene supported membranes of chromium ferrocyanide and cerium (IV) molybdate gels with different amounts of binding materials. They observed that specific conductance decreases with increasing the amount of binder. Neena Mahadevan and Mrs. Uma Sharma (145) explained the phenomenon of transport of alkali metal cations through liquid membrane using non-cyclic synthetic ionophores and discussed the relationship between the structure and transport ability.

Ken Iseki, Mitsura Sugawara, Nobutaka Saitoh (146) demonstrated the transport mechanism of organic cations across rat intestinal brush-border membrane.

A number of thoretical models (point charge model) and finite ion size model for the solid/electrolyte conductor interface have been used to interpret the impedance characteristics under various conditions of blocked, unblocked and partially blocked electrolyte depending upon the extent penetration of electrolyte to the electrode (116-119). Armstrong has attempted to use some of the theoretical models of aqueous electrolyte systems in order to obtain a simple model.

In majority of cases an electrochemical cell is better understood by a complicated net work of resistance and capacitance. These show a complex behaviour in the complex impedance plane. If an impedance spectrum is given, one can calculate the components of an equivalent circuit of resistance and capacitance responsible for it. Thus with the measurements an electrochemical cells it is useful for investigator to measure the impedance of the cell and subsequently to find the probable equivalent circuit and the significance of different components. This is usually carried out by comparing the results with the thoretical model.

CHAPTER - III

MATERIAL AND METHODS

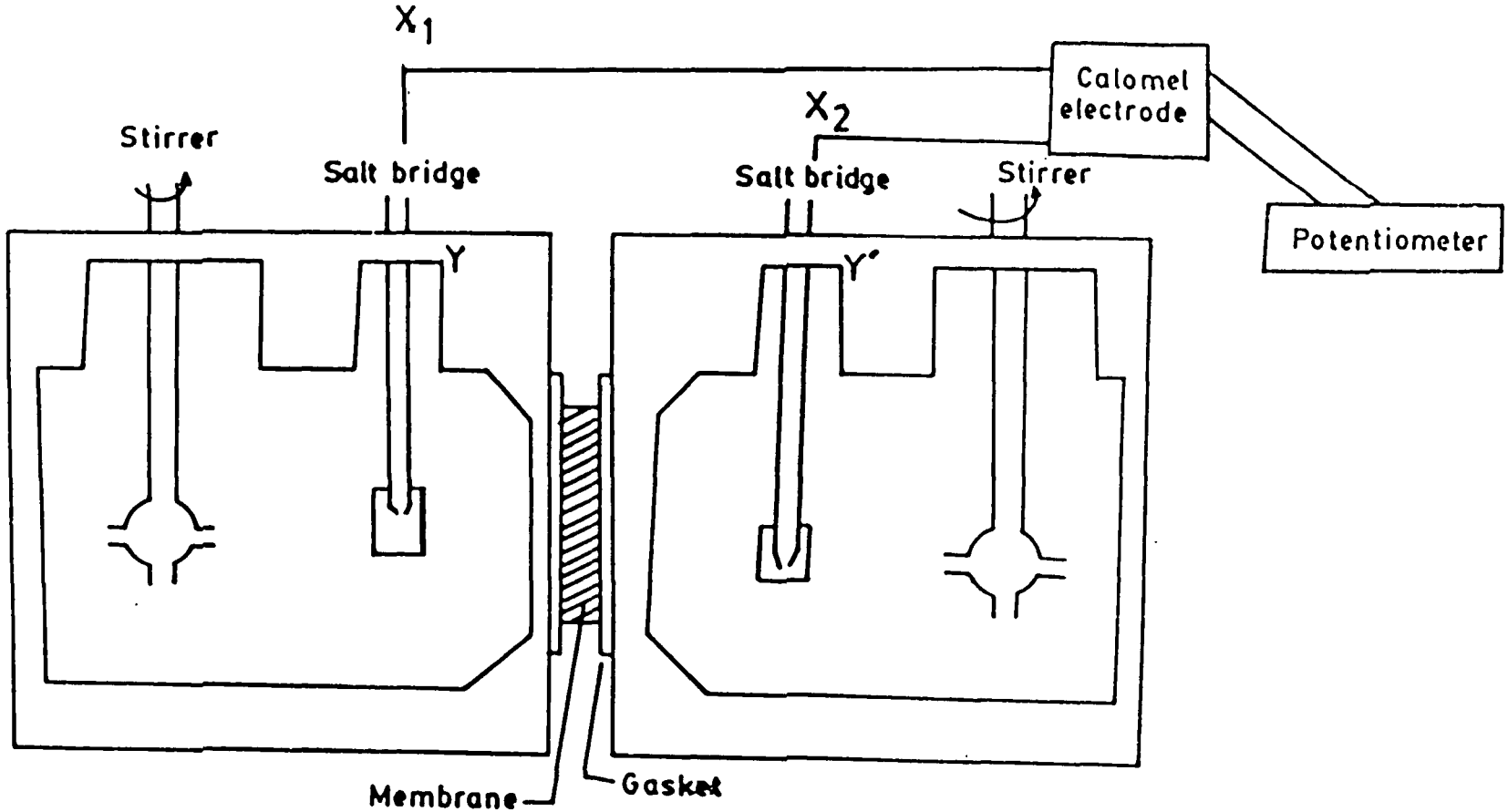


Fig.1 Schematic diagrame of cell used for the measurement of membrane potential.

EXPERIMENTAL:

The pericardial membrane was taken out immediately after slaughtering buffalo (Bof-bubalis) aged between 18-24 months, and immersed in ice-cold Ringer's sclution (147,148) of pH 7.4±0.2, for preservation of tissues.

RINGER'S SOLUTION:

To prepare Ringer's solution, 9 gms of sodium chloride (NaCl); 0.42 gms of potassium chloride (KCl); 0.24 gms of calcium chloride ($CaCl_2$), 1.0 gms of glucose ($C_6H_{12}O_6$) and 0.15 gms of sodium bicarbonate, salts were dissolved in double distilled water. Calcium chloride ($CaCl_2$) and glucose were dissolved in the solution just before the membrane was put into it.

APPARATUS AND EXPERIMENTAL METHODS:

The cell assembly used for the measurement of electrical resistance (R_E) and electrical capacitance (C_E) is shown in Fig. 1. It consisted of two half cells. The vertical female joints Y and Y' attached to each half cell provide for introducing the electrolyte solution and platinum electrodes X_1 and X_2. The cell was divided into two symetrical compartments by water tight membrane which was placed between the brism of these two cell parts. Both the solutions were stirred vigrously by a pair of magnetic stirrer.

MEASUREMENT OF MEMBRANE POTENTIAL:

Prior to observation the membrane was washed three times with deionised water to remove the traces of Ringer's solution and was placed between the brism of two half cells. Solution on both sides of the pericardial membrane were stirred vigrously. The cell assembly was immersed in the thermostate water bath maintained at 25±0.1°C with constant stirring. The cell of the type

Pt electrode	salt solution	membrane	salt solution	Pt. electrode
	C		C	

was used to measure electrical resistance (R_E) and capacitance (C_E).

The electrolytes employed were solutions of different concentrations of sodium chloride (NaCl) and potassium chloride (KCl), of analytical grade (B.D.H., India). Solutions were made in deionised water. The same electrolyte with different concentrations was taken on both the sides of the membrane. After each observation the membrane used was replaced by newone. The experiments were repeated a number of times to minimise error with freshly prepared electrolytic solutions. The values of electrical resistance (R_E) and capacitance (C_E) were observed with freshly obtained pericardial membrane for each electrolytic solution

and the observations were recorded with the help of universal L.C.R. bridge -921.

CHAPTER - IV

RESULTS AND DICUSSION

The transport occuring across the artificial membrane is a passive phenomenon i.e. it does not require any living force to control transport activities of metal ions. Transport is, therefore, totally governed by physical forces.

In contrast to the above phenomenon, transport through biological membranes depend upon active forces i.e. it can maintain gradients of metal and non-metal ion forces across the membrane. As a result a potential difference between inside and out side is maintained. This phenomenon is known as active transport. That both active as well as passive tansport occure across the biological membrane is well known(8-12).

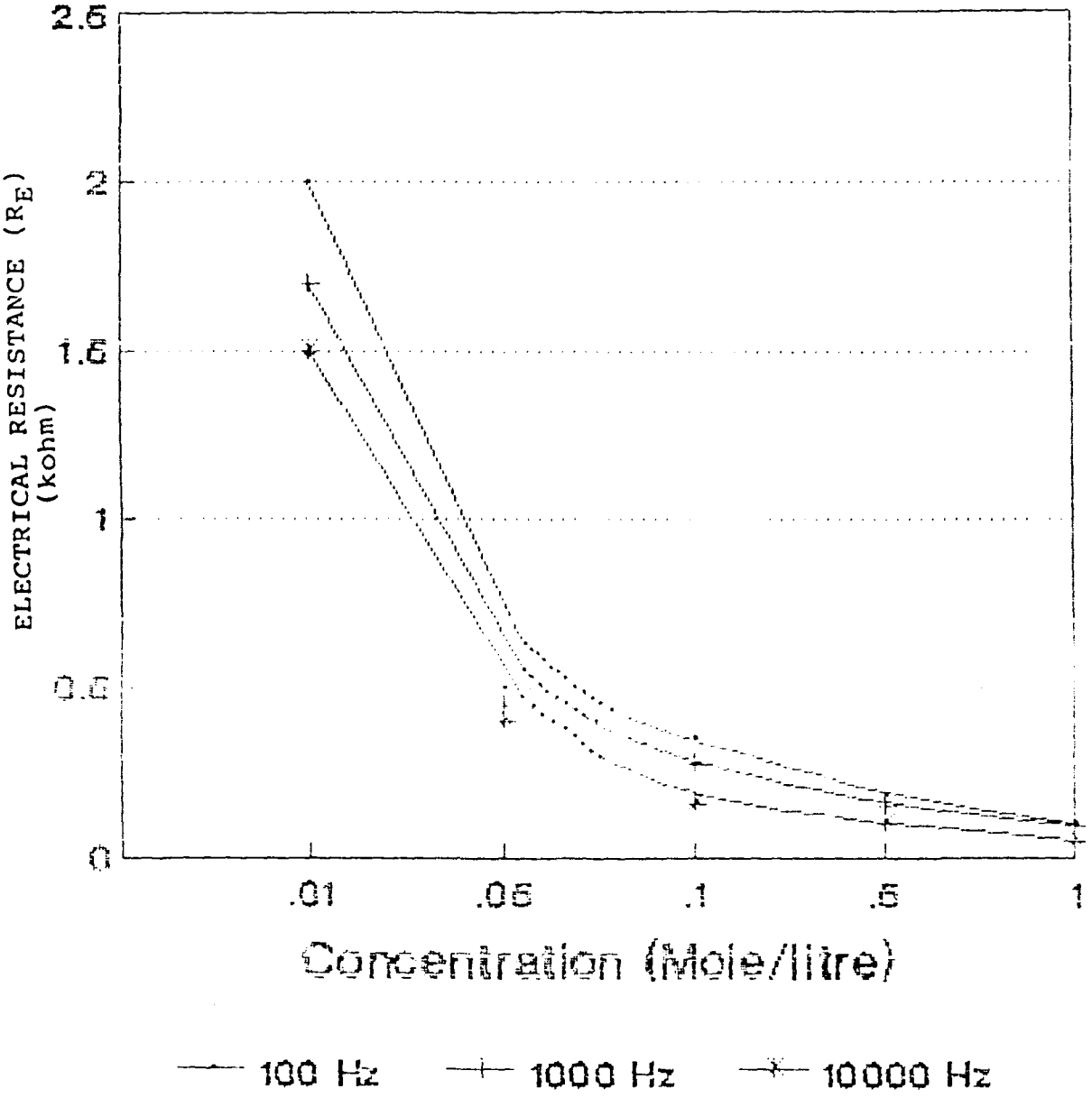
Arif et. al. (8-12) first reported that pericardium can form an easily accessible,simple and cheaper method for studying thermodynamical properties in biological system. The results have been confirmed in several papers by the same group of investigators. Because of the advantage of this model, the same is utilised to evaluate membrane resistance, capacitance, conductance and impedance by taking sodium chloride (NaCl) and potassium chloride (KCl) solution with different concentrations across pericardial membrane. The present study is, therefore, the first attempt to know the effect of concentration and frequency on the resistance and capacitance of pericardial membrane.

TABLE 1: Values of electrical resistance (R_E), observed across pericardial membrane, equilibrated with various concentrations of alkali chlorides (NaCl, KCl), at frequency ranging from $1x10^2$Hz to $1x10^4$Hz at 25±0.1°C.

ELECTROLYTE CONCENTRATION (mole/litre)	ELECTRICAL RESISTANCE (R_E) (kohm)					
	FREQUENCY 1×10^2Hz		FREQUENCY 1×10^3Hz		FREQUENCY 1×10^4Hz	
	NaCl	KCl	NaCl	KCl	NaCl	KCl
5×10^{-3}	-	7.400	-	6.800	-	4.000
1×10^{-2}	2.00	6.300	1.700	5.900	1.500	3.000
5×10^{-2}	0.500	5.000	0.4500	4.500	0.400	2.200
1×10^{-1}	0.350	4.000	0.280	2.200	0.160	1.300
5×10^{-1}	0.180	3.000	0.150	1.500	0.100	0.900
1×10^{0}	0.100	2.500	0.090	1.000	0.050	0.500

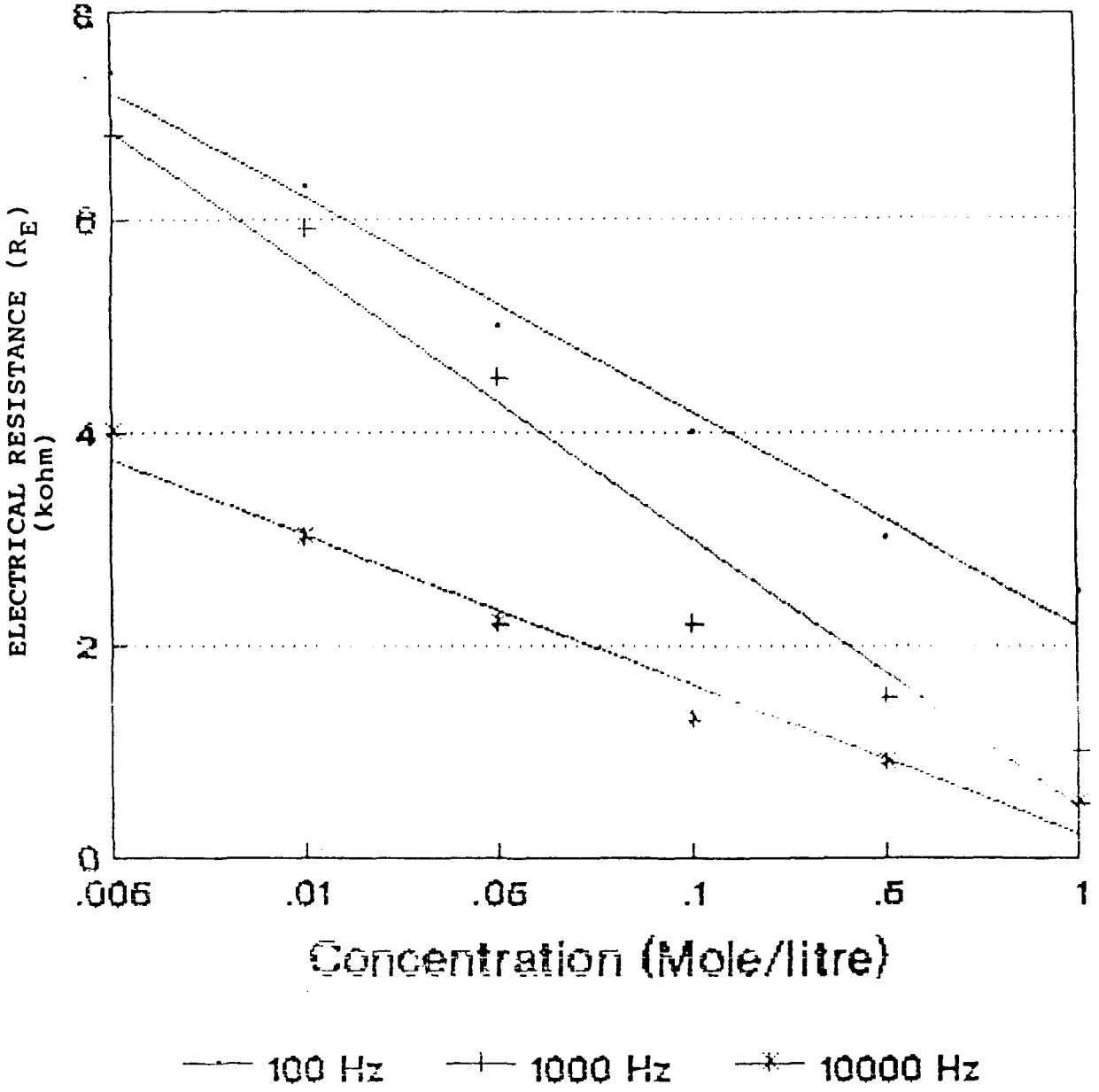
TABLE 2: Values of electrical capacitance (C_E), observed across pericardial membrane equilibrated with various concentrations of alkali chlorides (NaCl, KCl) at frequency ranging from $1x10^2$ to $1x10^4$Hz at 25±0.1°C.

ELECTROLYTE CONCENTRATION (mole/litre)	ELECTRICAL CAPACITANCE (C_E) (μF)					
	FREQUENCY 1×10^2Hz		FREQUENCY 1×10^3Hz		FREQUENCY 1×10^4Hz	
	NaCl	KCl	NaCl	KCl	NaCl	KCl
5×10^{-3}	-	0.00005	-	0.00015	-	0.0003
1×10^{-2}	-	0.00010	-	0.00020	-	0.0004
5×10^{-2}	0.0100	0.00013	0.0500	0.00025	0.0900	0.0005
1×10^{-1}	0.0200	0.00015	0.0900	0.00040	0.1900	0.0007
5×10^{-1}	0.0400	0.00030	0.1500	0.00065	0.2600	0.0009
1×10^{0}	0.0700	0.0010	0.2500	0.00260	0.7500	0.0035



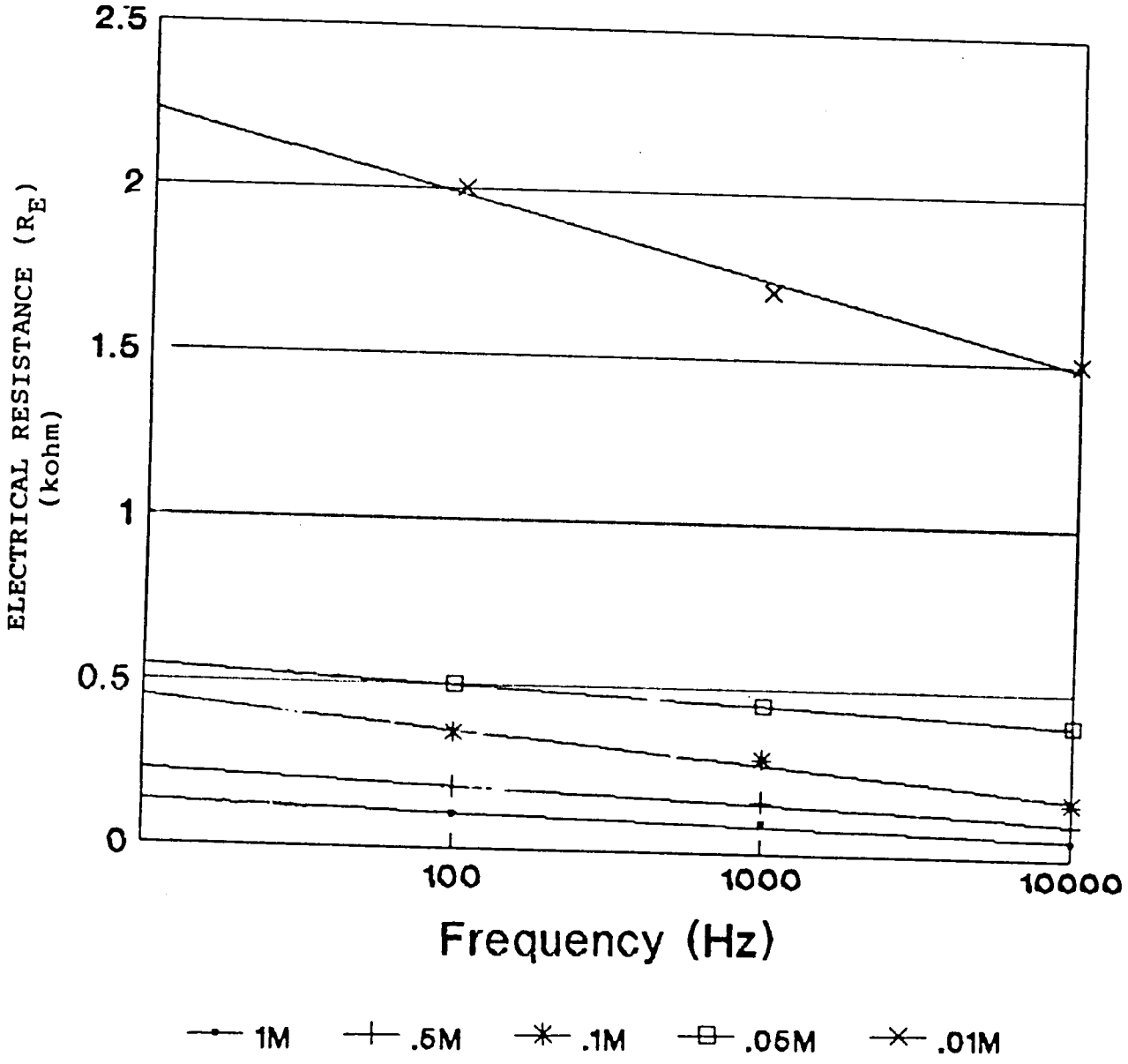
Plots of observed electrical resistance (R_E) against concentration for NaCl at different frequencies

Fig. 2



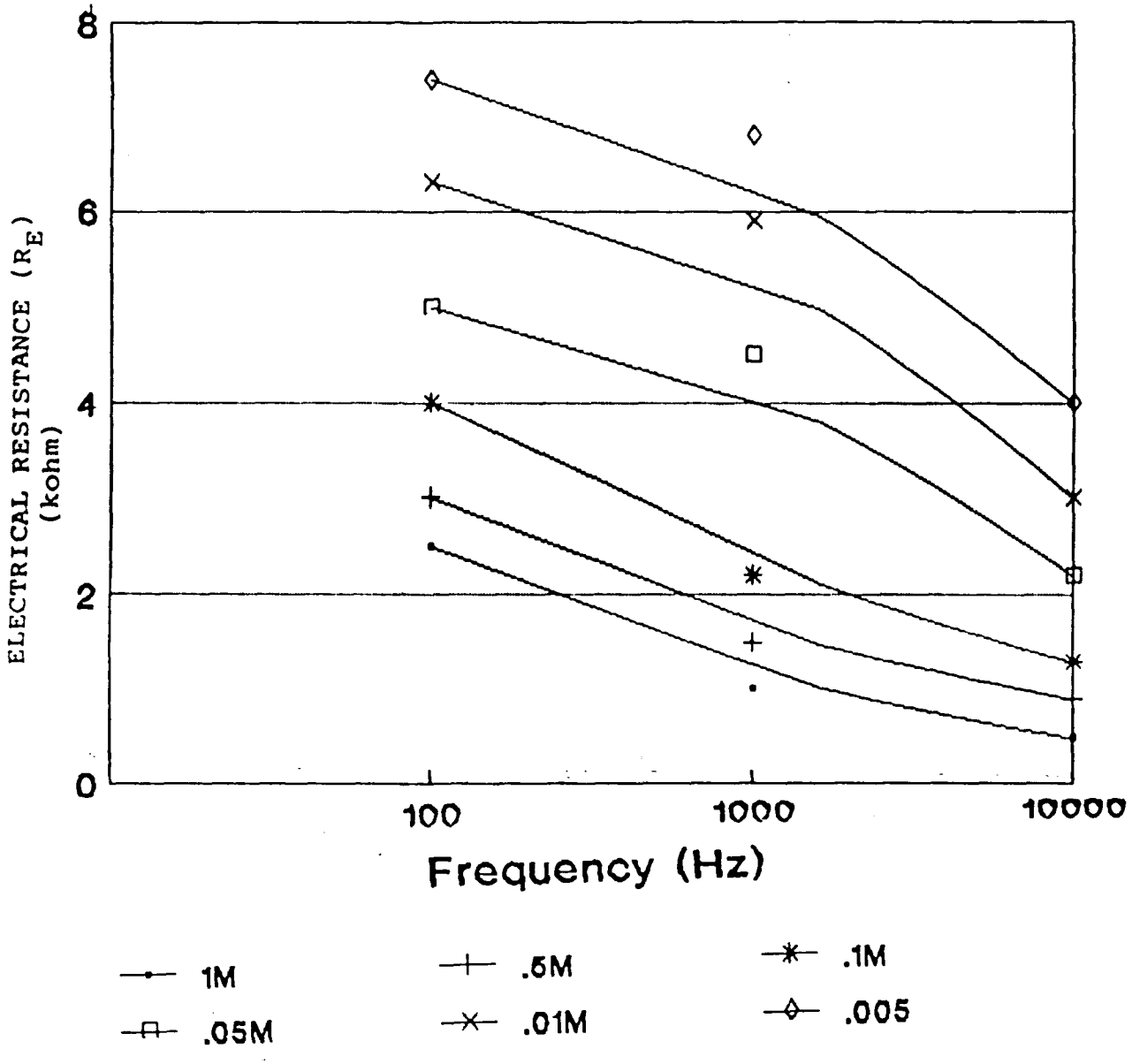
Plots of observed electrical resistance (R_E) against concentration for KCl at different frequencies

Fig. 3



Plots of observed electrical resistance (R_E) against frequency for NaCl at various concentration

Fig. 4



Plots of observed electrical resistance (R_E) against frequency for KCl at various concentrations

Fig. 5

The values of electrical resistance and capacitance across pericardial membrane, equilibrated with various concentrations of aqueous solutions of uni-univalent alkali electrolytes with common anion, over a frequency range $1x10^2$ to $1x10^4$ hertz have been measured. The concentration of electrolyte ranges from $1x10^{-3}$ to $1x10^0$ mole/litre. The values are shown in tables 1 & 2.

Plots between concentration verses electrical resistance as shown in fig. 3, indicates a linear decrease in electrical resistance with increase in concentration for KCl but a curved decrease in the value of electrical resistance for sodium chloride is observed fig (2). A sharp decrease in electrical resistance as concentration increases to $5x10^{-2}$ mole/litre and a slight decrease for further increase in concentration is shown. With the increase in applied frequency a slight linear decrease in the value of electrical resistance for NaCl and a slight curved decrease for KCl is observed (Figs. 4,5).

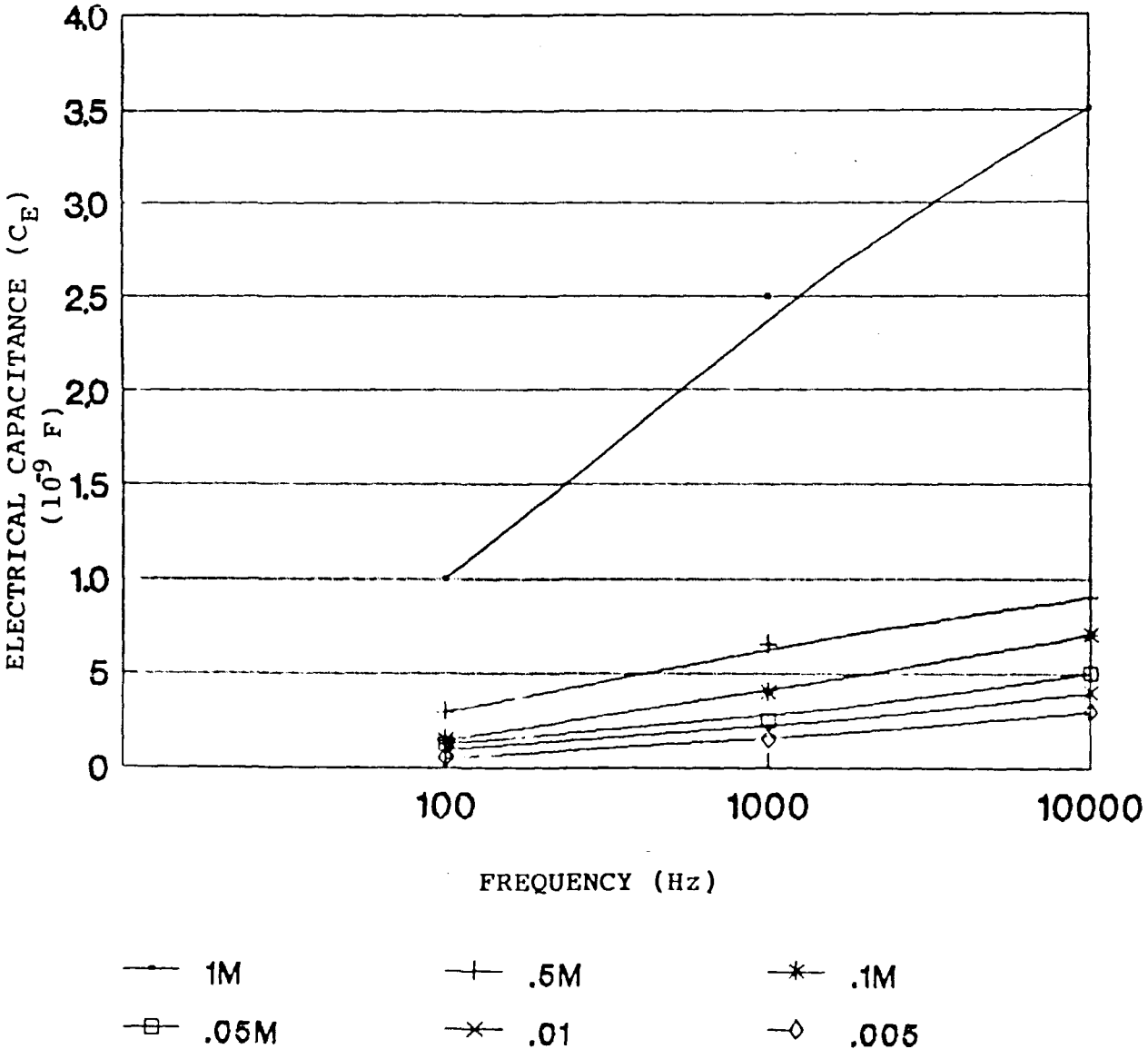
The decrease of electrical resistance with increase of concentrations of aqueous solutions of sodium chloride and potassium chloride and magnitude of applied frequency, are attributable, respectively, to increased electrolyte uptake and fast exchange of polarity resulting in a leakage of charge through the dielectric across the two surfaces of the membrane.

Different pattern of decrease in the electrical resistance, with the increase of concentration and magnitude of applied frequency, for the two electrolytes is observed. With the increase in concentration decrease in the electrical resistance for NaCl is more rapid than for potassium chloride. But with the increase in the magnitude of frequency, electrical resistance decreases more rapidly in case of KCl than NaCl. Observed electrical resistance of NaCl is less than KCl for entire range of frequency and concentration (Tables 1 & 2). The phenomenon can be well understood by the fact that K^+/Na^+ pump controls the active transport of ions across the biomembrane.

Experiments on nerve, muscles, frog skin, red blood cells, and cells from a number of other tissues have shown that Na^+ is transported from the cytoplasm to the interstitial fluid against an electrochemical gradient. But the definition given by Ussing (149), this is called active transport. A transport against an electrochemical gradient requires energy, and it has been shown by experiments on nerve (150,151) and on red blood cell membranes (152-154) that the energy for active transport of Na^+ comes from adenosine triphosphate (ATP). The active transport of Na^+ is dependant on concentration of K^+ in the extracellular fluid, and there seems to be some kind of a coupling between the active outward transport of Na^+ and inward transport of K^+ (154-157).

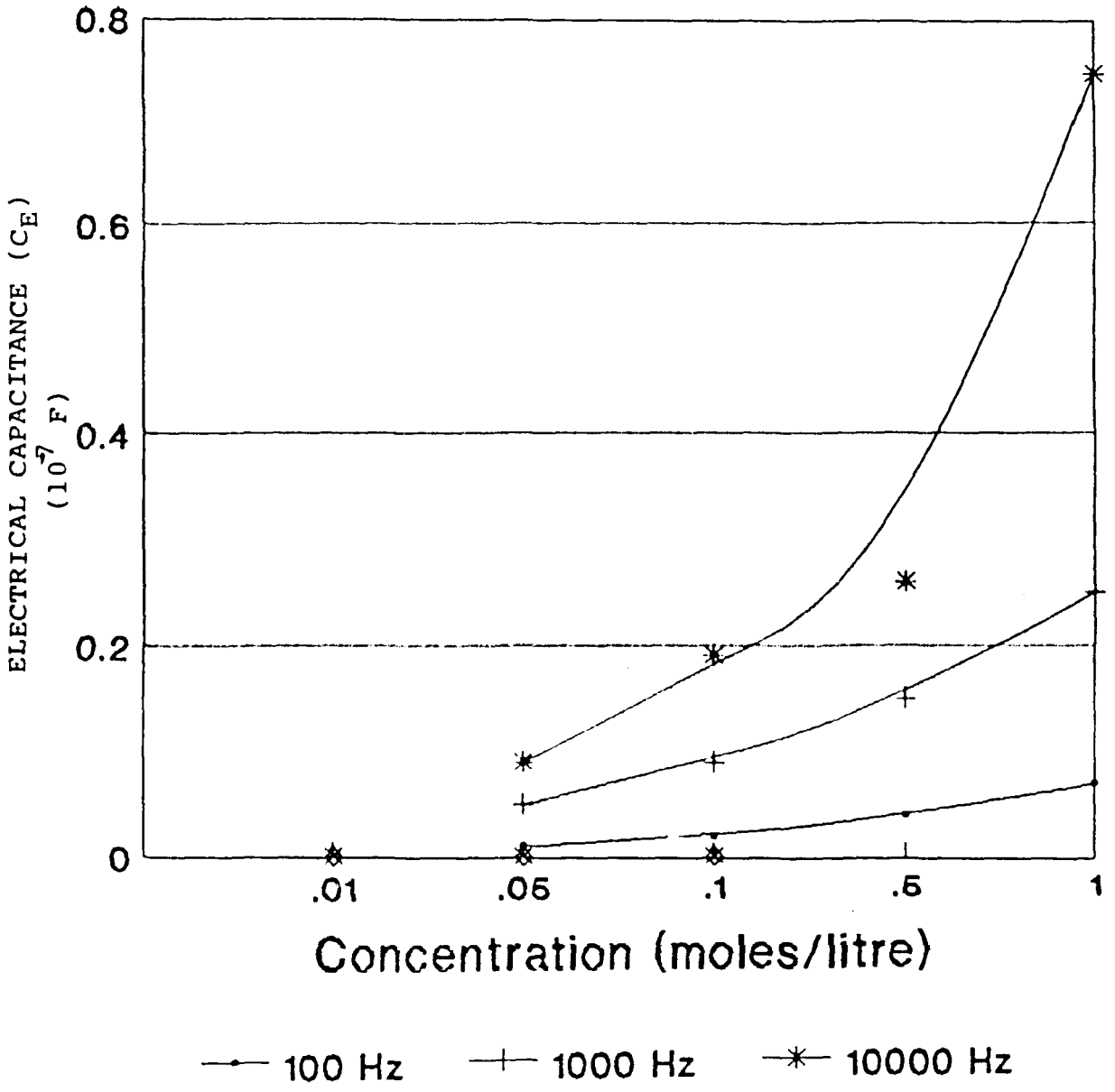
TABLE 7: Values of rectance (X), calculated from observed values of electrical capacitance (C_E), across pericardial membrane, equilibrated with various concentration of alkali chlorides (NaCl, KCl), at frequency ranging from 10^2 to 10^4Hz.

ELECTROLYTE CONCENTRATION (mole/litre)	REACTANCE (X) (kohm)					
	FREQUENCY 1×10^2Hz		FREQUENCY 1×10^3Hz		FREQUENCY 1×10^4Hz	
	NaCl	KCl	NaCl	KCl	NaCl	KCl
5×10^{-3}	-	318.20×10^2	-	106.06×10^1	-	53.03×10^0
1×10^{-2}	-	159.10×10^2	-	79.55×10^1	-	39.77×10^0
5×10^{-2}	15.91×10^1	122.38×10^2	3.18×10^0	61.19×10^1	1.76×10^1	31.82×10^0
1×10^{-1}	7.95×10^1	106.06×10^2	1.76×10^0	39.77×10^1	0.83×10^{-1}	22.72×10^0
5×10^{-1}	3.97×10^1	53.03×10^2	1.06×10^0	24.47×10^1	0.61×10^{-1}	17.67×10^0
1×10^0	2.27×10^1	15.91×10^2	0.62×10^0	6.36×10^1	0.21×10^{-1}	4.54×10^0



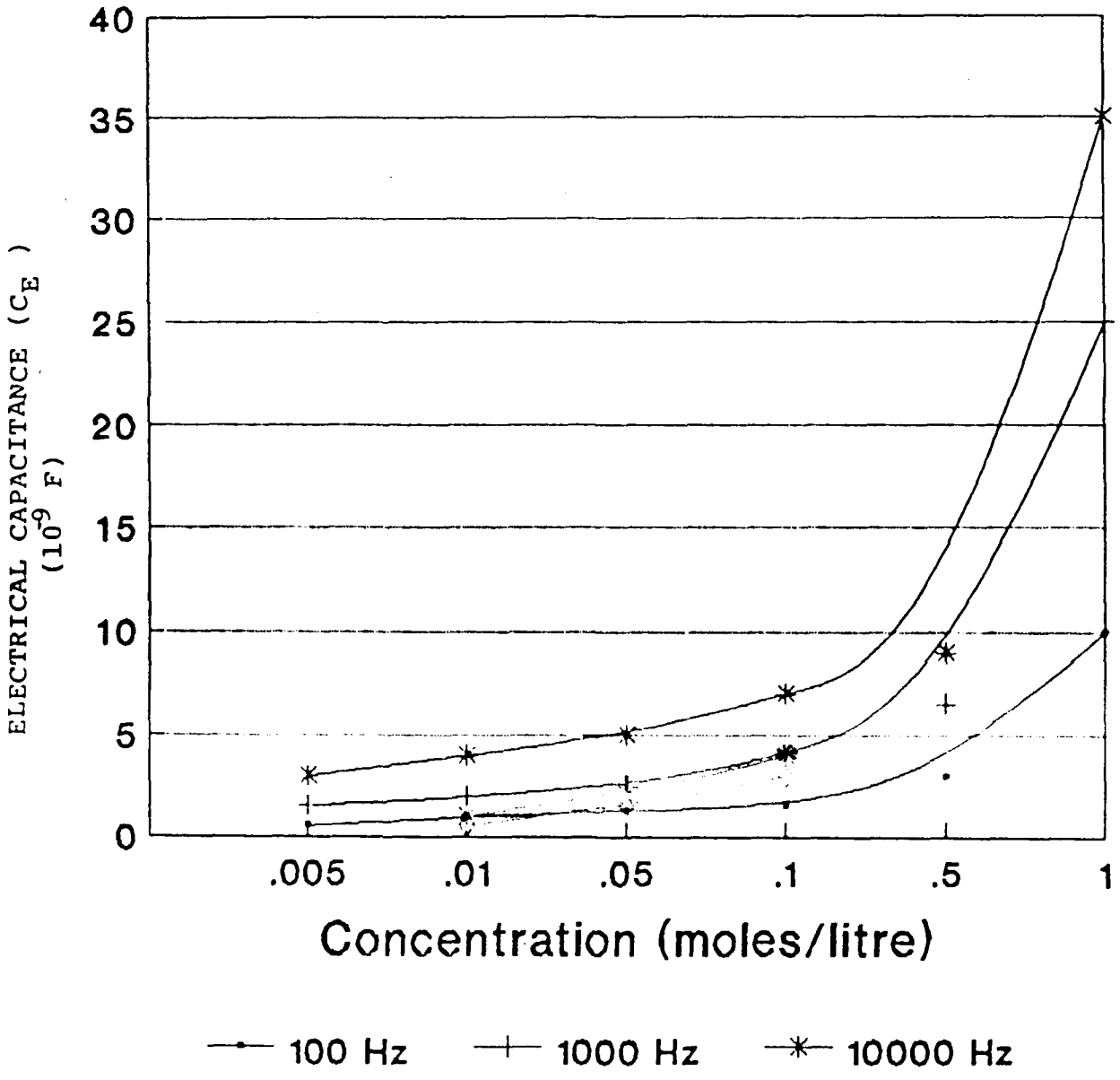
Plots of observed electrical capacitance (C) against frequency for KCl at various concentrations

Fig. 7



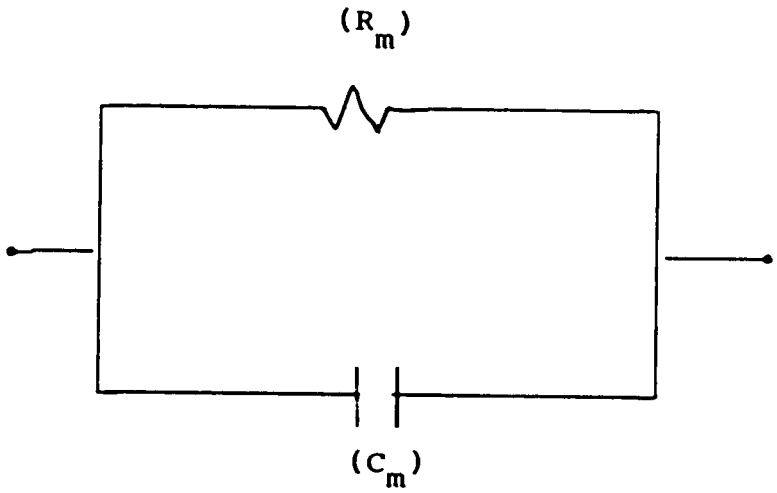
Plots of observed electrical capacitance (C) against concentration for NaCl at different frequencies

Fig. 8



Plots of observed electrical capacitance (C) against concentration for KCl at different frequencies

Fig. 9



ELECTRICAL CIRCUIT FOR A MEMBRANE/
ELECTROLYTE SYSTEM

Fig. 10

Figs. 6,7,8 & 9 show an increase in capacitance with increase in electrolyte concentration and frequency. At low concentrations the increase is nearly linear but at higher concentration a sharp increase is shown. This is due to the changes produced in the dielectric properties (ε) and the effective thickness (d) of membrane electrolyte system in accordance with the equation for parallel plate capacitor.

$$C_E = \varepsilon / 36 \pi \, 10^{11} d \qquad \ldots\ldots (1)$$

decrease in the value of 'd' is probably due to the deswelling of membrane because of the deswelling of water molecules from the membrane frame-work by the incoming ions.

In order to have a better understanding to the mechanism of flow of ions through the membrane, of the electrochemical properties and the electrical circuit asscciated with the system under investigation. Impedance, membrane resistance, and membrane capacitance have been evaluated on the basis of equivalent electrical circuit model (Fig. 10). For this circuit Laxminarayanaih and Shane (158,159) have propsed the following relationship

$$X = \frac{1}{wC_E} \qquad \ldots\ldots (2)$$

[$w = 2\pi f$, f is applied frequency used to measure R_E & C_E]

$$Rm = R_E \left[1 + \left(\frac{X}{R_E} \right)^2 \right] \qquad \ldots\ldots (3)$$

$$Cm = (X / R_E) \left(\frac{1}{w R_m} \right) \qquad \ldots\ldots (4)$$

TABLE 3: Values of membrane resistance (R_m), calculated from reactance (X) and observed values of electrical resistance (R_E), across pericardial membrane, equilibrated with different concentrations of alkali chloride (NaCl, KCl), at frequency ranging from $1x10^2$ to $1x10^4$Hz at 25±0.1°C.

ELECTROLYTE CONCENTRATION (mole/litre)	MEMBRANCE RESISTANCE (R_m) (kohm)					
	FREQUENCY 1×10^2Hz		FREQUENCY 1×10^3Hz		FREQUENCY 1×10^4Hz	
	NaCl	KCl	NaCl	KCl	NaCl	KCl
5×10^{-3}	-	$136.654x10^6$	-	$165.4480x10^3$	-	$707.124x10^0$
1×10^{-2}	-	$40.179x10^6$	-	$107.263x10^3$	-	$530.35x10^0$
5×10^{-2}	$50.62x10.^3$	$29.95x10^6$	$22.95x10^0$	$83.21x10^3$	$0.047x10^0$	$462.43x10^0$
1×10^{-1}	$18.08x10^3$	$28.10x10^6$	$11.34x10^0$	$71.92x10^3$	$0.020x10^0$	$398.67x10^0$
5×10^{-1}	$8.78x10^3$	$9.37x10^6$	$7.64x10^0$	$39.49x10^3$	$0.013x10^0$	$348.12x10^0$
1×10^0	$5.17x10^3$	$1.01x10^6$	$4.59x10^0$	$4.05x10^3$	$0.0058x10^0$	$41.82x10^0$

TABLE 4: Values of membrane conductance (C_c), calculated from membrane resistance (R_m), across pericardial membrane, equilibrated with various concentrations of alkali chlorides (NaCl, KCl) at frequency ranging from 10^2Hz to 10^4Hz.

ELECTROLYTE CONCENTRATION (mole/litre)	MEMBRANE CONDUCTANCE (C_c)					
	FREQUENCY 1×10^2Hz		FREQUENCY 1×10^3Hz		FREQUENCY 1×10^4Hz	
	NaCl	KCl	NaCl	KCl	NaCl	KCl
5×10^{-3}	-	0.073×10^{-10}	-	0.060×10^{-7}	-	0.141×10^{-5}
1×10^{-2}	-	0.248×10^{-10}	-	0.093×10^{-7}	-	0.188×10^{-5}
5×10^{-2}	0.197×10^{-7}	0.333×10^{-10}	0.435×10^{-4}	0.120×10^{-7}	0.209×10^{-2}	0.2162^{-5}
1×10^{-1}	0.553×10^{-7}	0.355×10^{-10}	0.881×10^{-4}	0.139×10^{-7}	0.490×10^{-2}	0.250×10^{-5}
5×10^{-1}	1.137×10^{-7}	1.067×10^{-10}	1.307×10^{-4}	0.253×10^{-7}	0.720×10^{-2}	0.287×10^{-5}
1×10^{0}	1.932×10^{-7}	9.876×10^{-10}	2.178×10^{-4}	2.468×10^{-7}	1.700×10^{-2}	2.390×10^{-5}

TABLE 5: Values of membrane capacitance (C_m), calculated from reactance (X), membrane resistance (R_m) and observed values of electrical resistance (R_E), across pericardial membrane, equilibrated with various concentrations of alkali chlorides (NaCl, KCl), at frequency ranging from $1x10^2$ to $1x10^4$Hz at 25±0.1°C.

ELECTROLYTE CONCENTRATION (mole/litre)	MEMBRANE CAPACITANCE (C_m) (μF)					
	FREQUENCY 1×10^2Hz		FREQUENCY 1×10^3Hz		FREQUENCY 1×10^4Hz	
	NaCl	KCl	NaCl	KCl	NaCl	KCl
5×10^{-3}	-	0.00005	-	0.00015	-	0.00029
1×10^{-2}	-	0.00010	-	0.00020	-	0.00040
5×10^{-2}	0.00900	0.00013	0.04900	0.00026	0.14650	0.00049
1×10^{-1}	0.02000	0.00015	0.08817	0.00035	0.4085	0.00060
5×10^{-1}	0.03990	0.00030	0.14700	0.00065	0.70830	0.00080
1×10^{0}	0.06994	0.00100	0.24510	0.00250	1.16300	0.00345

TABLE 6: Values of impedance (Z), calculated from reactance (X) and electrical resistance (R_E) across pericardial membrane, equilibrated with various concentrations of alkali chlorides (NaCl, KCl), at frequency ranging from 10^2 to 10^4Hz at 25±0.1°C.

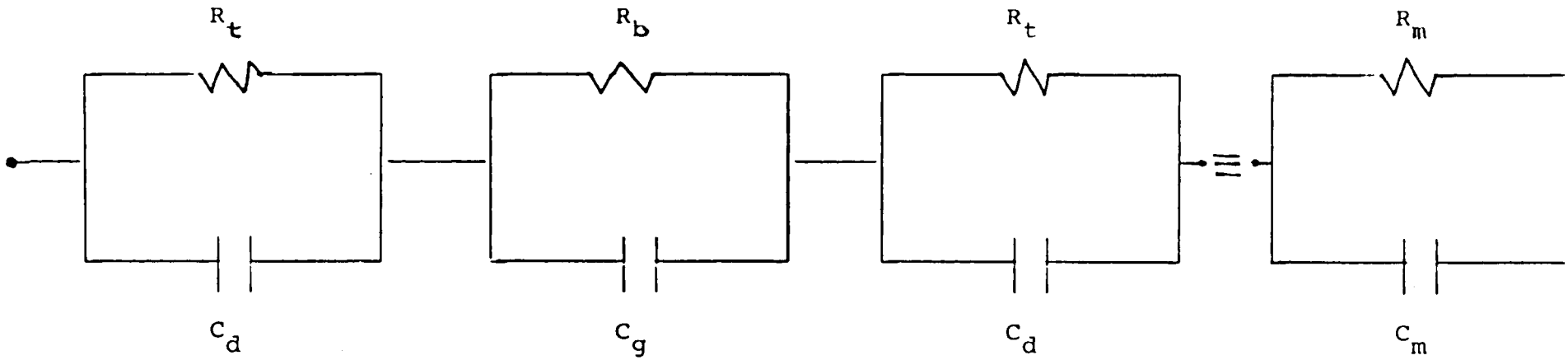
ELECTROLYTE CONCENTRATION (mole/litre)	IMPEDANCE (Z) (kohm)					
	FREQUENCY 1×10^2Hz		FREQUENCY 1×10^3Hz		FREQUENCY 1×10^4Hz	
	NaCl	KCl	NaCl	KCl	NaCl	KCl
5×10^{-3}	-	31.82×10^3	-	10.80×10^2	-	5.33×10^1
1×10^{-2}	-	15.91×10^3	-	7.95×10^2	-	4.008×10^1
5×10^{-2}	15.91×10^1	12.23×10^3	3.21×10^0	6.12×10^2	4.37×10^{-1}	3.207×10^1
1×10^{-1}	7.95×10^1	10.60×10^3	1.78×10^0	3.97×10^2	1.80×10^{-1}	2.274×10^1
5×10^{-1}	3.97×10^1	5.30×10^3	1.07×10^0	2.44×10^2	1.17×10^{-1}	1.770×10^1
1×10^0	2.27×10^1	1.59×10^3	0.64×10^0	0.63×10^2	0.54×10^{-1}	0.457×10^1

$$Z = (R_E^2 + X^2)^{\frac{1}{2}} \quad \ldots\ldots (5)$$

Where X is the reactance, R_E and C_E are the electrical resistance and capacitance observed for the model membrane equilibrated with different concentrations of uni-valent electrolytes. R_m and C_m are the membrane resistance and membrane capacitance. Z is the impedance.

The observed data (Tables 3,4,5 & 6) indicate that the values of R_m, membrance conductance (C_c), C_m and (Z) are the function of bathing electrolyte concentration. The fact may be ascribed due to progressive accumulation of ionic species within the membrane, thus making membrane more and more conducting, while decrease in membrane resistance (increase in membrane conductance) with increase of applied frequency may be due to the fast exchange of polarity resulting in a leakage of charge through the dielectric across two surfaces of the membrane.

Electrical double layer theory (124) may also be used to interpret the change produced in the magnitude of membrane capacitance (C_m) with the change in bathing electrolyte concentration. The electrical double layer at the membrane/solution interface has been utilised in several studies to account for various membrane behaviour (99,137). The polarising charge on the geometric capacitor in the form of diffused double layer plays an important roll and affects



EQUIVALENT ELECTRICAL CIRCUIT FOR A MEMBRANE/
ELECTROLYTE SYSTEM

Fig. 11

the overall membrane capacitance. The applied frequency across the membrane has been found to effect doubel layer capacitance by the movement of ions across it. In order to investigate impedance characteristics of the membrane/ electrolyte system and the double layer effect, the equivalent electrical circuit has been analysed further and is represented in fig. (11). This circuit represents a solid smooth surface in contact with penetrating electrolyte and refers to ideal impedance spectra on complex plane (121).

Impedance of the proposed equivalent electrical circuit (Fig. 11) for the membrane/electrolyte system is given by -

$$\frac{2Rt}{1+j\ w\ Cd\ Rt} + \frac{Rb}{1 + j\ w\ Cg\ Rb} = \frac{Rm}{1 + j\ w\ Cm\ Rm} \quad ...(6)$$

Where Cg is the specific geometric capacitance, which is assumed to depend upon the structural details of the polymer network of which the membrane is composed, Cd is the interfacial electrical double layer capacitance, Rb is the bulk resistance of the membrane, and Rt is the charge transfer resistance between membrane/electrolyte interface assuming the ion transfer process to be single step.

Real and imagenary parts of the above equation (6) are given by -

TABLE 8: Evaluated values of interfacial double layer capacitance (C_d), across pericardial membrane.

ELECTROLYTE CONCENTRATION (mole/litre)	INTERFACIAL DOUBLE LAYER CAPACITANCE (C_d) (μ F)					
	FREQUENCY 1×10^2 Hz		FREQUENCY 1×10^3 Hz		FREQUENCY 1×10^4 Hz	
	NaCl	KCl	NaCl	KCl	NaCl	KCl
5×10^{-3}	-	0.000105	-	0.000319	-	0.000657
1×10^{-2}	-	0.000220	-	0.000434	-	0.000904
5×10^{-2}	0.020650	0.0002988	0.122950	0.000580	0.335200	0.001170
1×10^{-1}	0.056000	0.000352	0.276400	0.000814	1.259300	0.001450
5×10^{-1}	0.180000	0.000857	0.360000	0.001756	3.623000	0.002080
1×10^{0}	-	-	-	-	-	-

$$\frac{Rm}{1 + w^2Cm^2\ Rm^2} = \frac{Rb}{1 + w^2Cg^2Rb^2} + \frac{2Rt}{1 + w^2Cd^2Rt^2} \quad ...(7)$$

$$\frac{Cm\ Rm^2}{1+w^2Cm^2\ Rm^2} = \frac{Cg\ Rb^2}{1 + w^2Cg^2\ Rb^2} + \frac{2\ Cd\ Rt^2}{1 + w^2\ Cd^2\ Rt^2} \quad ...(8)$$

At higher oscillator frequencies equation (8) can be approximated as -

$$\frac{1}{Cm} = \frac{1}{Cg} + \frac{2}{Cd} \quad .... (9)$$

Equation (9) indicates that the membrane/electrolyte system may be considered to be composed of three capacitors arranged in series. Geometric capacitor is placed between the two interfacial double layer capacitor as suggested by Armstrong. For high electrolyte concentration and/or significant surface charge (124, 160)

$$\frac{1}{Cg} >> \frac{2}{Cd}, \text{ so that, } \quad Cm \approx Cg$$

Taking this value of Cm as Cg at 1M NaCl & 1M KCl solutions different values of Cd at other electrolyte concentrations are calculated by using equation (9). It is found that the value of Cd, for both the electrolytes, increases with increase in electrolyte concentration, thereby pointing towards the dependence of Cm on Cd.

Cm should differ considerably from Cg when -

$$\frac{1}{Cg} \approx \frac{2}{Cd}.$$

This situation prevails in the absence of surface charge at low electrolyte concentrations.

The exact form of double layer capacitance depends upon the fixed charge (σ_s) and the membrane potential (Vm). If $\sigma_s = 0$, then,

$$Cd = \frac{\varepsilon_o \varepsilon_w \ \mathrm{Sinh}\alpha}{(1/k)\alpha} \qquad \ldots\ (10)$$

where $\varepsilon_o = 8.85 \times 10^{-14}$ F/Cm, ε_w is the dielectric coefficient of water, α is a constant which takes into account the structural details of membrane polymer; and (1/k) is the Debye-Huckel length, given by -

$$\frac{1}{k} = \frac{4.31 \times 10^{-8}}{(2 \times \mu)^{\frac{1}{2}}} \qquad \ldots\ (11)$$

'μ' is the ionic strength of bathing electrolyte soloution. 'α' is determined from the transcedental equation -

$$\left[\frac{\varepsilon_o \varepsilon_w}{(1/k)Cg} \ \mathrm{Sinh}\alpha + 2\right] = \frac{V_m}{2(RT/F)} \qquad \ldots\ (12)$$

or alternatively from -

$$Cm \cdot Vm = \sigma_p = 4\ FC\ (1/k)\ \mathrm{Sinh}\alpha \qquad \ldots\ (13)$$

where σ_p is the polarisation charge on capaciter. Equation (10) can be reduced to

$$Cd = \frac{\varepsilon_o \varepsilon_w}{(1/k)} - \qquad \ldots\ (14)$$

If Vm << RI/F, so that $\mathrm{Sinh}\alpha = \alpha$, the value of Cd calculated

TABLE 9: Values of electrical double layer capacitance (C_d), calculated from equations '9' & '14'.

ELECTROLYTE CONCENTRATION mole/litre	C_d FROM EQUATION '14' (uF)	C_d FROM EQUATION '9' (NaCl) (uF)	C_d FROM EQUATION '9' (KCl) (uF)
5×10^{-3}	16.400	-	0.00065
1×10^{-2}	23.600	-	0.00100
5×10^{-2}	51.906	0.3352	0.00117
1×10^{-1}	73.000	1.2593	0.00145
5×10^{-1}	164.000	3.6230	0.00280
1×10^{0}	232.00	-	-

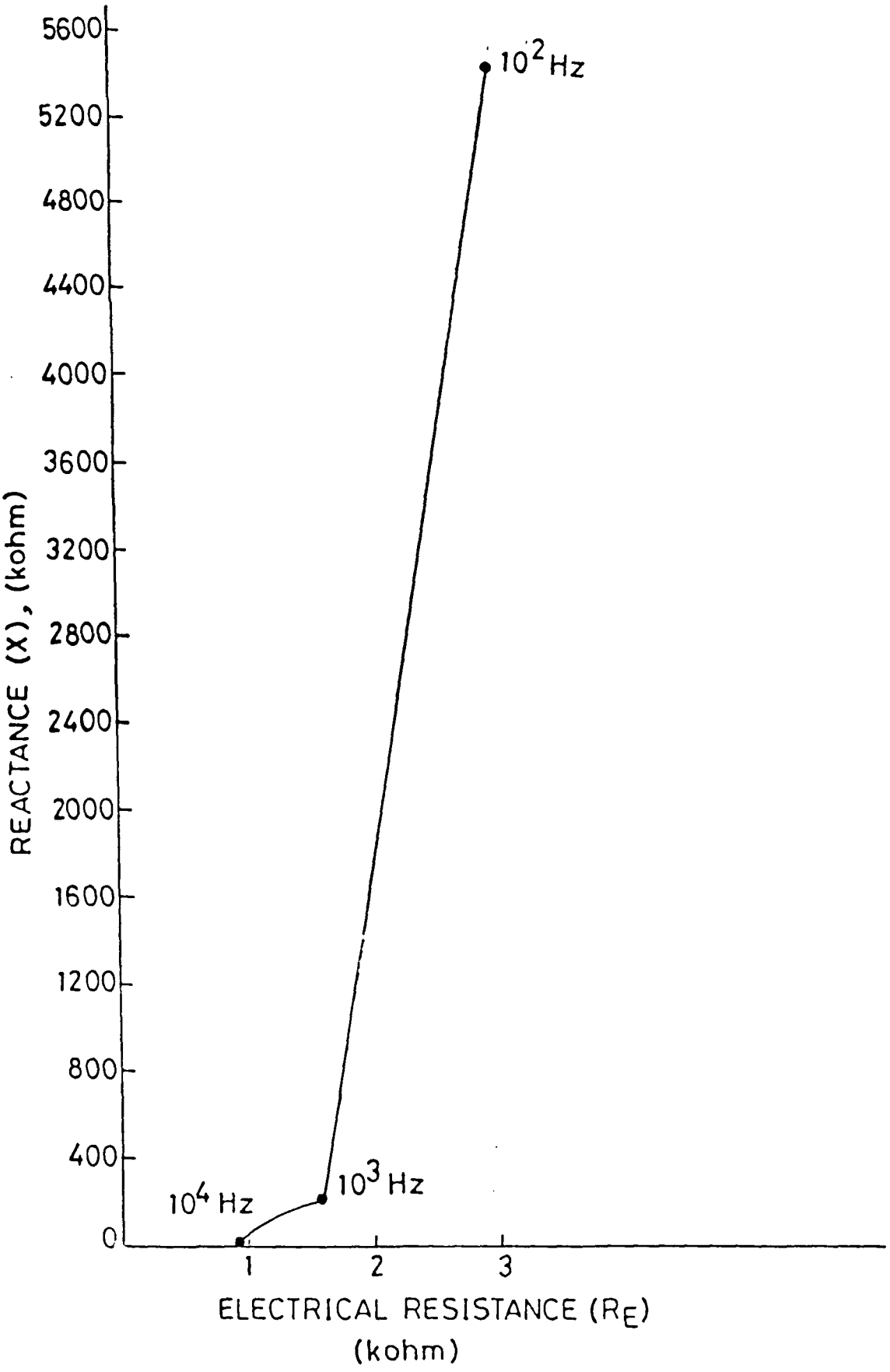


Fig. 12: Plots of reactance (X) against electrical resistance (R_E) for pericardial membrane equilibrated with $5x10^{-1}$M KCl solution.

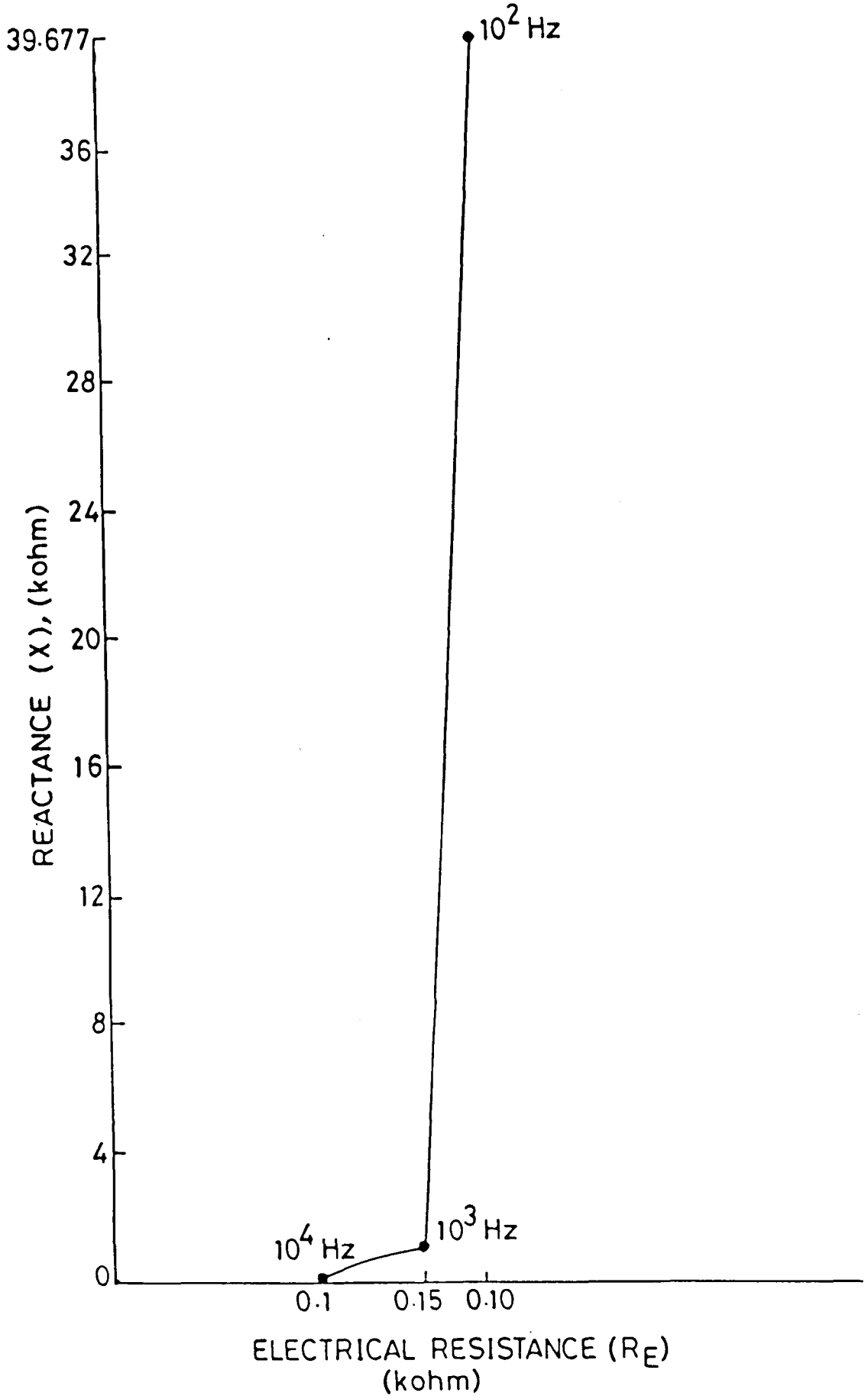


Fig. 13: Plots of reactance (X) against electrical resistance (R_E), for pericardial membrane, equilibrated with $5x10^{-1}$M NaCl solution.

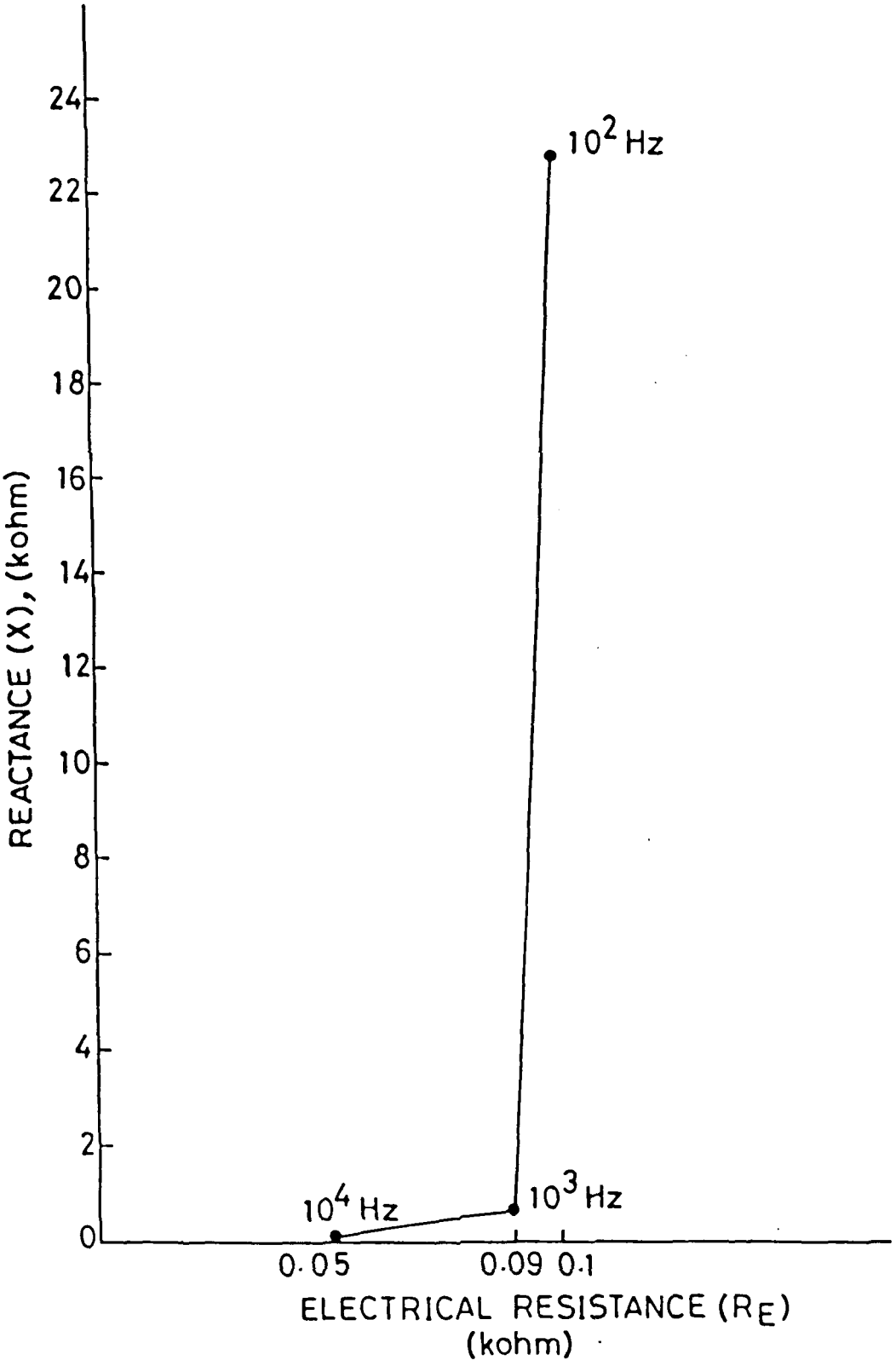


Fig. 14: Plots of reactance (X) against electrical resistance (R_E) for pericardial membrane equilibracted with $1x10^0$M NaCl solution.

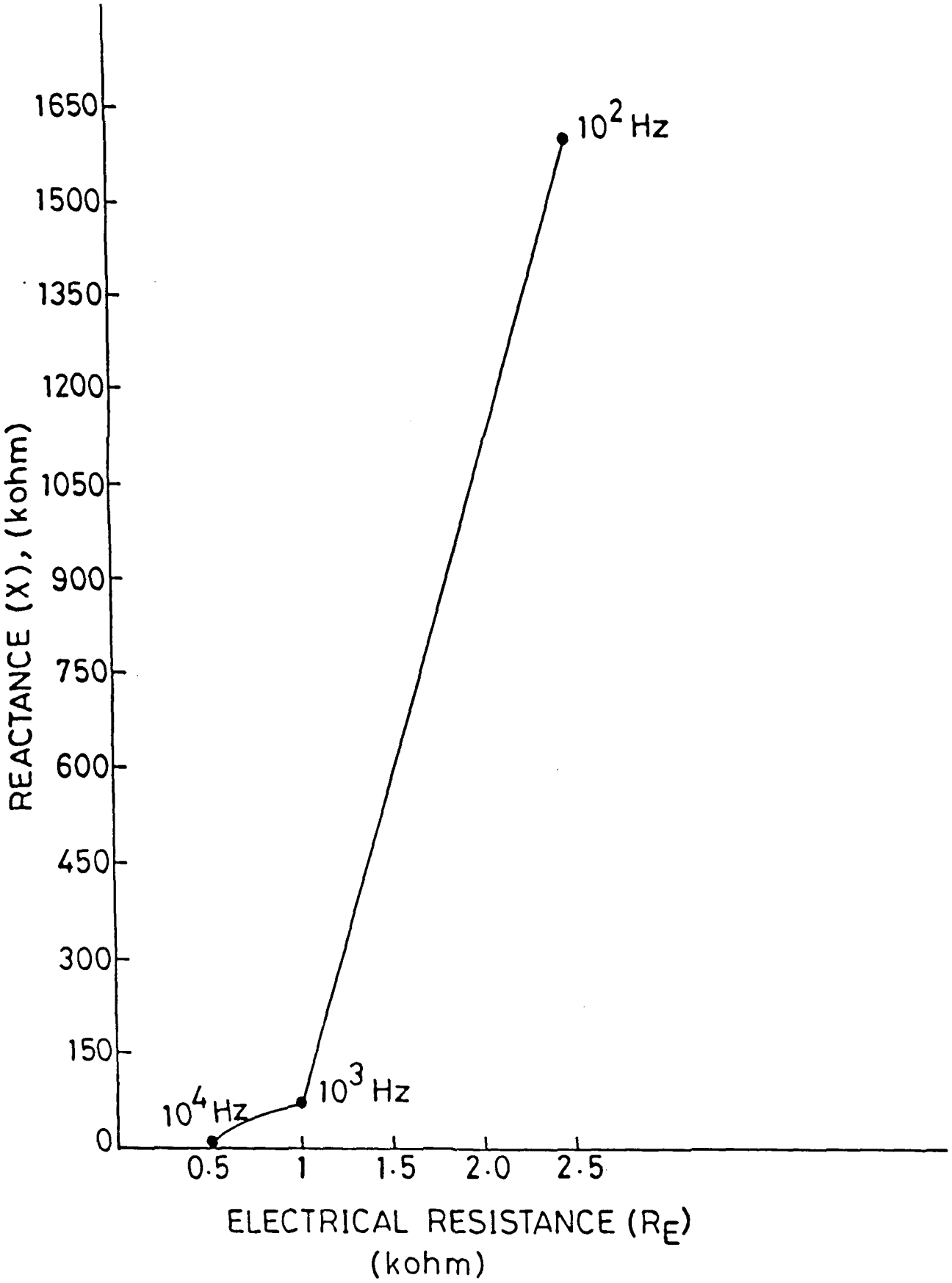


Fig. 15: Plots of reactance (X) against electrical resistance (R_E), for pericardium equilibrated with $1x10^0$M KCl solution.

from eq (14) at different electrolyte concentration are given in table (9). The difference in the values of Cd calculated from eq (9) and (14) is attributed to the presence of polarisng charge and other structural details of membrane matrix.

It has been found that the value of 'Cd' increases with increase in electrolyte concentration due to decrease in the thickness of interfacial double layer (Debye-length) and/or occumulation of ions at the membrane solution interfaces.

The equivalent electrical circuit model used for present investigation represents a solid smooth surface in contact with the penerating electrolyte refers to the ideal impedance spectra in the complex plane in the form of a semicircle (129). The evaluated data follow the theoretical prediction at higher frequencies (Figs 12, 13, 14 & 15). The deviation at lower frequencies may be attributed to non-homogeneous nature of pericardial membrane.

FUTURE PLAN OF WORK

Some of the properties which can be utilised to determine the energy characteristic of a cell membrane such as fixed charge density, permselectivity, enthalpy,entropy, free energy associated with reversible and irreversible process of thermodynamics, energy of activation etc. are therefore of great importance in elucidating this aspect of cell membrane characteristic in normal and disease states.

Because of the great clinical significance of biological membranes it should be explored in great depth. Keeping this in view our further plan of work is to elucidate the effect of disease states on chemical composition and electrophysiological properties of membrane.

Thermodynamic properties of cells are highly important because energy of activation and other thermodynamic features will determine the transport across the cell. The energy content of the cell also determines its biological activity. The cell multiplication and degree of neoplastic activity have been found to be influenced by the energy content of the milignant cell. Studies on thermodynamic of simple ion diffusion i.e. free energy of activation, energy of activation, entropy of activation and enthalpy of activation will be performed both in normal and in disease states. These parameters will be evaluated by the method developed by Kittle-Berger (13). Membrane

Resistance (Rm), capacitance (Cm), inductance (I) and impedance (Z) will be observed both in normal and disease states by the methods developed by several investigatons.

Further plan of work is to study the influence of various drugs such as diureties which alters the trace metal composition of body fluids, on the biophysical behaviour of these membranes and to examine biochemical and biophysical properties of the membrane in animals like rabbit, dog and monkeys both in normal and in disease states. To produce disease like diabetes, uremia and anaemia in these animals is easier.

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